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BIOPHYSICS

Institut d'Estudis Catalans, Barcelona, Catalonia
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CONTRIB SCI 11:225-235 (2015)
doi:10.2436/20.7010.01.234

Neuronal cultures: The brain's complexity and non-equilibrium physics, all in a dish

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Summary. Neuronal networks—and the brain in particular—are out of equilibrium systems in which neurons are the interacting elements. These cells are coupled through physical connections and complex biochemical processes. Moreover, they are capable of self-organization and shape a rich repertoire of spatiotemporal patterns and dynamic states. An elegant yet powerful experimental tool to investigate and describe the features of neuronal networks is neuronal cultures, in which neurons are extracted from brain tissue, dissociated, and cultured in an appropriate environment. Here we introduce the difficulties in understanding the complexity of the brain and its dynamics. We then present the fundamental concepts—from a statistical and non-linear physics viewpoint—needed to describe neurons and networks. These concepts lay the foundations needed to discuss recent models of brain dynamics. We then introduce neuronal cultures, highlighting their enormous potential as accessible and controllable living neuronal networks. Finally, we show how neuronal cultures, and their physical modeling, constitute a remarkable platform to investigate fascinating questions in the non-equilibrium physics of the brain and to provide new insights to advance the treatment of neurological disorders. [**Contrib Sci** 11(2):225-235 (2015)]

Approaching the brain's complexity through model systems

Understanding the brain is not only a scientific curiosity. It is the path to understanding ourselves, our social behavior, and the modification of both upon the brain's malfunction. This quest recently fostered the establishment of two grand international brain enterprises, the "Human Brain Project" [23,47] and the "BRAIN Initiative" [19,23,47], supported, respectively, by the European Commission and the US government. The scope of these projects, "a bold new research effort to revolutionize our understanding of the human mind" in President Obama's words, is to bridge basic neuron-to-neuron interactions with brain function and cognition, which could ultimately shed light on new approaches to the treatment of neurological disorders, one of the largest burdens of our aging society. The two projects consider different yet complementary strategies: the "Human Brain Project" aspires to build a meticulous computer simulation of the human brain, while the "BRAIN Initiative"

Keywords: connectivity · neuronal culture · network models · noise · spontaneous activity

has targeted the development of new technologies aimed at the simultaneous monitoring of all neurons in the brain.

Although both actions have a collaborative vision and facilitate interdisciplinary research, the scientific community has recently raised serious concerns as to the validity of some of the proposed methodologies and their goals. For instance, if the "Human Brain Project" is based on a simulation of 1011 neurons and 1014 connections, how sensitive would it be to the simulation parameters themselves? Or, would the observed activity patterns reflect actual brain functions?

The debate surrounding these grand projects points out the importance of tackling the brain using different scales and approaches, to first comprehend general mechanisms before investigating the precise molecular details. Indeed, the brain exhibits a number of reliable features (such as widespread rhythmic activity or synchronization across distant areas) that suggest the involvement of robust mechanisms. These must be sufficiently stable in the presence of perturbations or fluctuations in the biochemical environment, but sufficiently flexible to allow for the processing of information and to respond to stimuli. In this context, two fundamental questions arise: How can these mechanisms be investigated in an accessible manner? What principles govern the emergence of collective behavior in neuronal networks?

Reducing the brain's complexity to unveil general mechanisms has both experimental and theoretical aspects. Experimentally, one can start by analyzing living neuronal networks of gradually larger size and richness. The nematode worm *Caenorhabditis elegans* and the freshwater fish *Danio rerio* (zebrafish) constitute the two most well-explored "simple" model organisms used in brain research. *C. elegans* has 302 neurons spread along its body and its set of 7000 connections (connectome) has been mapped out in its entirety [41]. Recent experiments have managed to simultaneously record the activity in all of the worm's neurons [27] and to even perturb the activity of some of them. Zebrafish have their neurons organized in a brain; although its precise connectome is still not fully drawn, in recent experiments the activity of most of the 100,000 neurons of a larval-stage zebrafish were simultaneously recorded [1]. In addition to these highly valuable model organisms, the need for systems that can be more readily controlled and accessed has put neuronal cultures in the frontline of tunable (and living) complex systems [31]. Indeed, the small size of neuronal cultures, as well as their preparation in controlled environments, has greatly facilitated their manipulation, monitoring, and analysis.

Theoretically, the brain—and its "simplified" analogs—can be approached by developing models with different levels of physico-mathematical complexity and biological accuracy, depending on the scale of the system under study and the particular problem to be addressed. Indeed, the neuronal assembly and its set of input and output connections configure a network whose ultimate dynamic traits depend on three major agents: the neurons themselves (intrinsic neuronal firing properties), the layout of connections (connectivity), and the inherent fluctuations in this biological system (noise).

The beauty of nonlinear physics is that it provides remarkable tools to describe these agents and to delineate their operation and mutual interaction. These interacting agents are then used to reproduce the characteristics of the observed activity patterns, predict their behavior, expose universal mechanisms, and even uncover hidden processes. The resources at hand are extensive and include: (i) the use of biophysical models to describe neurons [16,21]; (ii) graph-theoretical tools from statistical physics and mathematics to describe the connectivity map [4,5]; (iii) dynamic systems approaches to render the organization and stability of activity patterns [22]; and (iv) fluctuation theory to account for the effects of noise [12,24,30].

Neuronal cultures

Neuronal cultures [11,25] are typically prepared by first isolating a fragment of neuronal tissue from a specific brain region, for instance, the hippocampus or cortex of embryonic mice or rats. The neurons are then dissociated and seeded over culturing substrates such as glass (Fig. 1A), effectively establishing within a few days a *de novo* network rich in spontaneous activity. The size and shape of the culture can be controlled by different means (such as hollow masks, Fig. 1A), which allows the establishment of multiple cultures in the same well and thus their simultaneous access (Fig. 1B) [26]. These types of preparations are the expertise of our laboratory in Barcelona [26,36,37].

A detail of a typical neuronal culture is shown in Fig. 1B. Neurons appear as spherical objects whose connections are so dense and entangled that they cannot be resolved. Measuring activity in these cultures is obviously the first step towards understanding their modus operandi. Two main techniques are used to record neuronal activity: fluorescence calcium imaging [17] and electrodes [33]. Calcium techniques (Fig. 1C,D) are based on the use of fluorescent probes to detect the influx of calcium ions upon neuronal firing. Active neurons appear as bright

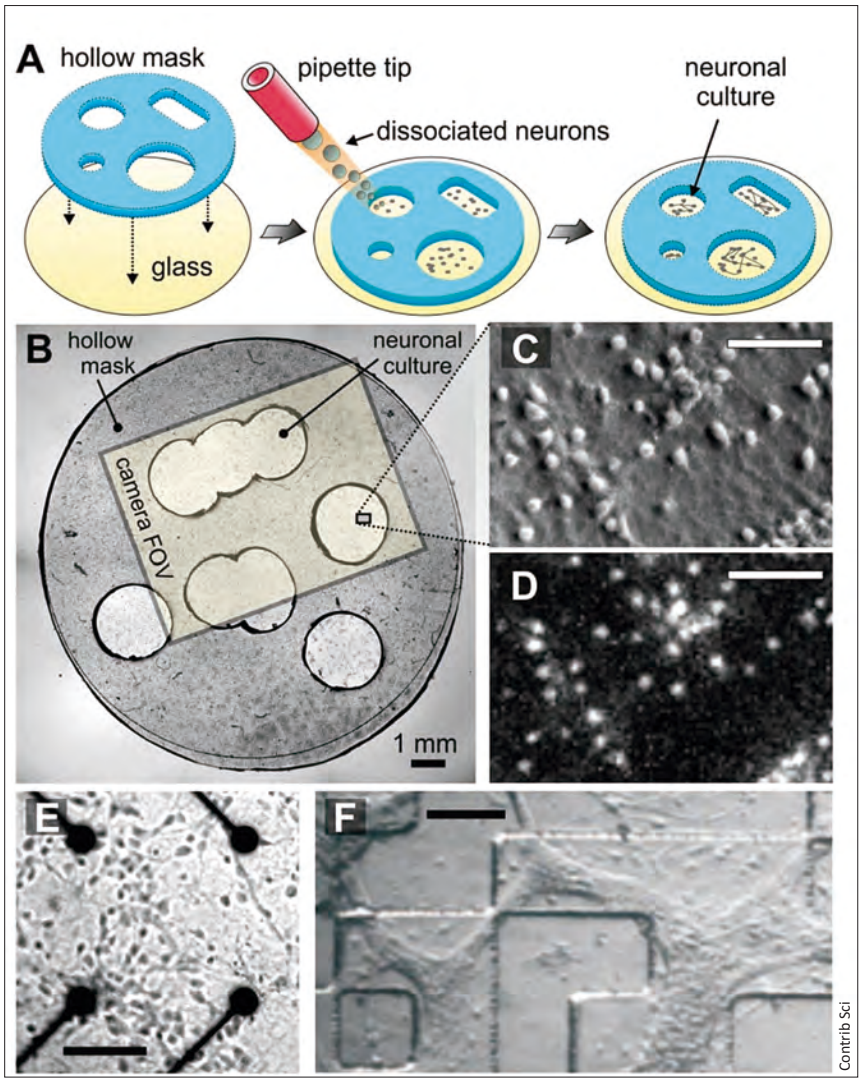

Fig. 1. Neuronal cultures. (**A**) Schematic representation of the culture protocol. Neuronal growth is supported by a substrate (typically glass), which may be accompanied by a hollow mask or other structure to control the size and shape of the network. Dissociated neurons are homogeneously plated over the substrate in the presence of chemicals that support their development and then cultured for several days. After 1–2 weeks in vitro, the neurons have reconnected and shaped a new network with rich spontaneous activity. (**B**) A single glass coverslip 13 mm in diameter may easily contain a single network with ~10^5 neurons, or a number of small networks with a typical density of 1000 neurons/mm^2. A fluorescence camera attached to an optical system can access in its field of view one or more small cultures. (**C**) Detail of a small region of a culture. Individual neurons appear as circular objects 10 µm in diameter. (**D**) Corresponding fluorescence image. Bright spots are firing neurons. (**E**) Neurons cultured over a substrate that contains a grid of electrodes (black structures in the image), which directly record the electrical activity of the neurons. (**F**) Patterned culture in which neurons grow at the crevices of a mold. The scale bars in C–F are 100 µm. (Figures adapted from [11,26]).

spots whose fluorescence intensity is proportional to the number of firings elicited by the neurons. Electrode-based techniques (Fig. 1E) directly measure the electrical activity of neurons. Although the latter methods offer higher temporal resolution and sensitivity, the number of neurons that can be accessed is limited by the number of electrodes, which impedes the study of large networks. Calcium imaging, by contrast, is limited only by the optical recording system. Current technology has made possible the simultaneous recording of thousands of neurons, both in vitro [26,37] and in vivo [1].

Neuronal cultures prepared under conditions in which the neurons uniformly cover the substrate and connect equally in any direction are termed homogeneous (Fig. 1C–E). Conversely, patterned cultures are those in which the position of the neurons and their connectivity are in some way dictated, thus allowing for complex configurations or neuronal circuits with specific characteristics [13,45]. One of

the many possibilities is the use of topographical molds (Fig. 1F), in which neurons grow along either the bottom or the top of a two-level pattern, giving rise to a highly anisotropic and inhomogeneous culture.

In general, the interest of patterned cultures for Physics and Neuroscience is twofold. On the one hand, they allow the design of simple circuits with known properties from which basic questions, such as the propagation of activity [14,15] and information coding [13,14], can be investigated. On the other hand, and within a more general perspective, patterned networks allow the controlled study of activity-connectivity relationships. This "engineering" procedure is not possible in intact, native brain tissue since the major structural paths are genetically dictated and therefore hardwired. Patterning in cultures adds guidance and strong spatial anisotropies, effectively shaping different connectivity layouts and activity patterns.

Spontaneous activity in cultures

Examples of recorded activity in neuronal cultures are shown in Fig. 2. In a homogenous culture (Fig. 2A), each neuron is selected as a region of interest to extract its fluorescent trace, i.e., the brightness of the neuron over time. As depicted in Fig. 2B, the fluorescence intensity averaged over the population (3000 neurons in this particular preparation) is characterized by quasi-periodic episodes of high activity combined with silent intervals. By inspecting each neuronal trace, one observes that these activity events encompass all the neurons (Fig. 2B, yellow box), shaping what is known as a "network burst." The analysis of these bursts reveals several very interesting features of the network and the mechanisms that control spontaneous activity [26], a problem that we address in more detail below. The fluorescence traces also reveal sporadic, asynchronous firing events (red arrowheads). These firings are also of interest, since they convey information on single neuron-to-neuron interactions that possibly reflect a direct physical connection between those neurons [35].

In patterned cultures, the inclusion of strong inhomogeneities in the connectivity induces very different dynamics. A simple yet informative preparation—and the focus of research in our laboratory [36]—consists of shaping aggregates of neurons that connect to one another (Fig. 2C, top). Each aggregate can be treated as an "effective neuron," which greatly reduces the number of dynamic elements in the network (Fig. 2C, bottom). These networks exhibit spontaneous activity patterns with rich spatiotemporal variety (Fig. 2D). Additionally, some of the connections between aggregates are directly visible, which makes the system a very attractive one to study the interplay between activity and connectivity, or as a model system to investigate network resilience to damage [36].

Neuron models

A neuron can be viewed as a "black box" able to receive inputs from other neurons, finally generating an output if the number of received inputs within a short time window is sufficiently large. The key variable for the representation of a firing neuron is the membrane potential, which changes according to whether the inputs are excitatory or inhibitory. The former increases the membrane potential, while the latter decreases it.

The timing between inputs, their strength, and their ultimate integration by the membrane are important aspects that shape neuronal responses and the dynamics of the network. It is precisely the complex processing of the neuronal inputs, as well as the on/off nature of the outputs, that confers a neuron with its nonlinear behavior.

Describing a neuron in detail is a mathematically difficult task, and a serious challenge when thousands of neurons are coupled together to assemble a meaningful network. However, what often interests physicists and neuroscientists is the collective action of the networks, in which case a very detailed biological description of the individual neurons is not necessary. This allows the use of relatively simple models with phenomenological parameters adequate to the scale and characteristics of the system under study [11,21,26].

The most popular and intuitive model is the "integrate-and-fire" (IF) model [21], in which the membrane potential increases with the number of inputs until it reaches a threshold, at which point the neuron "fires" (i.e., generates an action potential) and the membrane potential is reset to the resting state. The action potential travels along the axon to finally become the input of the connected neurons. The IF model uses a single equation and four parameters and can be easily modified and extended to reproduce sufficiently well the behavior of most neurons. One of these modifications defines the so-called Izhikevich model [20,21], which includes an additional variable that accounts for the recovery of the membrane potential, giving rise to a set of two coupled differential equations with four parameters.

A significant jump in biological detail (and mathematical

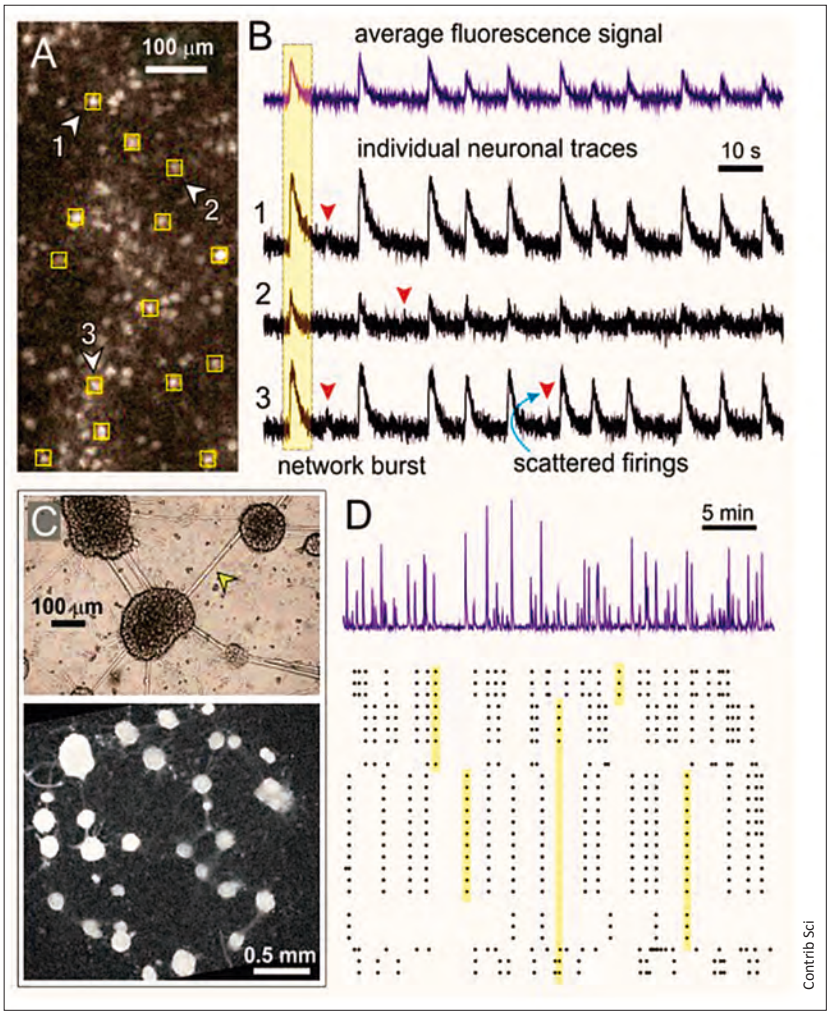

Fig. 2. Fluorescence imaging and neuronal network activity**.** (**A**) Fluorescence image of a small region of a homogeneous neuronal culture. Each neuron (bright spots) is associated with a region of interest (square box) from which time traces of neuronal activity are extracted. (**B**) The analysis of spontaneous activity in a typical experiment provides the average fluorescence signal (corresponding to the activity of ~1000 neurons) and all the individual single neuron traces. The yellow box depicts a network burst; the red arrowheads indicate scattered neuronal firings. (**C**) Patterned culture formed by interconnected islands of tightly packed neurons. Top: a detail of the network; bottom: a fluorescence image of the entire network during activity. The yellow arrowhead indicates a connection between clusters. (**D**) Corresponding network spontaneous activity. Top trace: the average fluorescence signal of the network along the recording; bottom plot: the activity of the network, with each dot indicating the occurrence of a firing in one of the islands. Neuronal islands tend to fire in groups with rich variety in the number of participating islands (yellow boxes). (Figures adapted from [36,37]).

complexity) is obtained with the Hodgkin-Huxley model [18], which describes the variations in the membrane potential together with the activation or inactivation of sodium and potassium currents. This model was introduced already in 1952 to explain the generation and propagation of action potentials in the axon of the giant squid. The model was easily adapted to different neural systems, and thus within a short time became a fundamental tool in theoretical and computational neuroscience. Indeed, its developers were awarded the Nobel Prize in Physiology or Medicine. The model consists of four main equations and tens of parameters which, when properly adjusted, allow the reproduction of all the different forms of spiking neurons [21]. However, the complexity of the model has made it impractical for studying systems with a large number of neurons, and therefore the simpler models outlined above—which are actually reduced versions of the Hodgkin-Huxley model—are largely used, despite the loss of detail. Because collective phenomena are dominant in large neuronal assemblies, simple neuronal descriptions suffice to remarkably well reproduce the major dynamic traits of a neuronal network (e.g., the spontaneous activity patterns shown in Fig. 2B [26]).

Network models

Like the streets and traffic lights in a city, the layout of neuronal connections and the adequate balance between excitation and inhibition are crucial for the normal operation of brain circuits. In epilepsy, for instance, an imbalance towards an excess of excitation brings the brain to a state of abnormal synchronization that totally disrupts its operability.

The description of the connectivity of networks has significantly advanced in the last two decades due to the enormous progress made in Network Science, which studies the statistical properties of complex networks in systems as diverse as the Internet, social relations, protein interactions, transportation, and neuronal tissues [4,5]. The identification of nodes and links is easy, for instance, in the case of airports and air routes, but when approaching the brain and neuronal networks it becomes more complicated.

On the one hand, in neuroscience a link can be structural or functional. Structural links are the actual physical connections between the neurons, while the functional ones are statistical correlations between the activity patterns of any two neurons. For instance, the activity traces of Fig. 2B show that neurons #1 and #3 fire together systematically and are therefore strongly correlated, i.e., they are linked in a functional manner. Since functional links reflect the flow of information across the neuronal circuit, they must be related to the underlying structural connectivity. How similar the functional network is to the structural one is an elegant problem and an entire research topic by itself [28].

On the other hand, the ability to identify nodes and links greatly decreases with the size of the network under study. A few neurons in a dish can be well monitored and their structural connections can even be resolved to some extent. This type of experiment, performed in our laboratory in Barcelona, can aid in uncovering the relation between structure and function [36]. At the scale of the brain, however, its sheer size and the limits of instrumentation impose the use of parcellations that contain thousands or millions of neurons. These parcellations are then the nodes, while correlations between those parcellations upon their activity define the corresponding functional links. Although the exact connectivity map between the neurons in the brain is unknown, there is a wealth of data describing major structural paths and interconnections between brain areas [38,44]. Again, the comparison of these maps may provide very important information and greatly help in understanding the brain and its alteration by various diseases.

The fundamental property that describes a given network, whether structural or functional, is the degree distribution $p(k)$, i.e., the probability that a node has k connections. The properties of this distribution delineate the important features of the system that it represents. For instance, let us consider a "toy network" in which a set of neurons are deposited over a flat surface. With no restrictions or guidance of any kind, all neurons will essentially connect to their neighbors, in which case the resulting $p(k)$ distribution is close to a Gaussian distribution, with a mean indicating the typical average connectivity of a neuron in the network and a width that reflects the inherent variability in the number of connections across neurons.

A signal generated at one end of this network will advance towards the other end in several steps, since it has to pass locally from neuron to neuron. This process can be well studied in the framework of statistical physics using concepts from percolation theory and criticality [7,32]. Conceptually, a network percolates if there is at least one path of connected neurons that bonds both ends of the network. If the neurons are highly connected, there will be several of these paths. The number of possible paths will rapidly diminish if the connectivity decreases. At a particular value of connectivity, percolation will no longer be possible, effectively breaking the circuit apart into small, disconnected islands. This value of connectivity defines a critical point that separates the connected from the fragmented layouts, and its study from an experimental perspective can shed light on interesting features of the structure and resilience of a neuronal network [32].

The toy network introduced above can be explored further to tackle other powerful concepts. If a few neurons in this network are allowed to form connections with very distant ones, then not only does the distribution of connections change, but the dynamics and percolative aspects are entirely reshaped. These "shortcuts" advance the information much faster and even synchronize a large number of neurons. They confer upon the network a "small-world" property [43], meaning that any neuron can be connected to any other through relatively few steps. *C. elegans* is an example of a known living neuronal network exhibiting this feature [39,41].

Again using this toy network, one can now imagine that neurons with these long-range connections have many more connections than other neurons and serve as true "hubs" that route information flow. These hubs may connect to one another to shape a structural core (or "rich club," in network language) that provides robustness to the network in the case of a random failure of nodes. Of the 302 neurons

of *C. elegans*, 11 have a much higher connectivity than the rest and form this type of structural core [39]. The failure of these neurons compromises the functionally of the entire system, while the loss of any other neuron causes relatively small damage since the core holds the network together.

This example provides just a glimpse of the potential of network theory. The true virtue of network theory, however, is that with minor modifications its theoretical framework can be applied to very different systems. In particular, it has revolutionized in just a decade our view of the healthy and diseased human brain [2,34,40]. The "small world" property, for instance, is believed to enable optimal cognitive functions at a low wiring cost, while the existence of "hubs" has been ascribed to efficient neuronal signaling, the integration of information, and communication. The alteration or loss of these topological traits in the brain has been extensively investigated, as it accounts for the damage in disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis.

Noise in neuronal systems

Noise is a remarkable example of a paradigm shift in neuroscience. Fluctuations initially considered as undesired were finally appreciated as a fundamental mechanism in the dynamics of neurons and neuronal circuits [24]. Generally speaking, noise can be viewed as the random fluctuations inherent in any physical system with a large number of degrees of freedom. In neuronal systems, at molecular and cellular scales, thermal fluctuations or variations in the biochemical environment often suffice to produce spontaneous neuronal firings that can propagate and be amplified throughout the network. At a macroscopic level, these firings shape the network noise, i.e., trains of low-amplitude random spikes that bombard neurons and circuits.

An illustrative case of the benefits of noise is "stochastic resonance" [46]. A neuron that receives a subthreshold input that varies in time (for instance in the form of a sine wave) cannot fire. However, the addition of noise of appropriate amplitude might induce firing just at the peak of the subthreshold signal. Since noise is ubiquitous in the network, the existence of a "resonator" greatly facilitates the amplification of small signals, and their detection. This is indeed the mechanism that the brains of predators use to detect very small perturbations in the environment and that signal the approach of a prey [29].

Stochastic resonance is just one of the several mechanisms in which the participation of noise is fundamental. For this reason, there is a tendency nowadays to introduce the more general term "stochastic facilitation" [24] to account for all possible mechanisms through a unified framework. Experiments and theoretical models show that, even in the absence of a periodic subthreshold signal, stochastic facilitation not only enhances the firing of neurons but also facilitates the generation of precisely timed (clock-like) trains of spikes, the synchronization of large populations of neurons, and the generation of oscillatory activity across brain areas. Precise timing and synchronization are pivotal since they confer reliability to key brain circuits, for instance, those involved in stimuli processing or motor coordination. Reliability is also a fundamental ingredient in the coding of information, its representation, and its recall through memory [12].

The stochastic facilitation framework is grounded in important physical concepts from dynamic systems theory, which by themselves have greatly helped to understand the exquisite dynamic repertoire of neuronal networks [42]. An example is the "attractor" concept, in which the dynamics of a neuronal network evolve towards a bounded number of states without much sensitivity to the initial conditions. At the other extreme, "chaotic" neuronal circuits are those with high sensitivity to the initial conditions; their dynamics are highly variable and unpredictable. Indeed, a fascinating feature of the brain is that some neuronal circuits exhibit striking reliability while others function at the verge of chaos. Chaotic operation seems to facilitate a quick response to stimuli [42] and may even drive complex cognitive tasks such as imagination.

The importance of spontaneous activity and its modeling

Living neuronal networks are active. Although this may seem obvious, a common and remarkable feature of all living neuronal networks is their rich dynamics, evoked by external stimuli or occurring spontaneously. Neuronal network dynamics range from the scattered firing of a few neurons to massive synchronous activations, giving rise to waves of activity or sustained oscillations with broad spatiotemporal characteristics. In the mammalian brain, correlated activity covers temporal scales of roughly 4 orders of magnitude, typically from 2 ms to 20 s, and encompasses a few tens to millions of neurons [6]. Additionally, the interplay between intrinsic neuronal dynamics and circuit connectivity is so

flexible and versatile that several patterns of activity can coexist within the same network. The repertoire of rhythms and their relative importance also depend on the state of the brain, i.e., at rest, sleeping, or performing specific tasks, and reflect the intimate relation of these rhythms to brain function, actually linking single-neuron activity to behavior.

Within the broad dynamic repertoire of the brain, spontaneous activity is a central feature [3,6]. This activity is not a "trivial random activity," as assumed for decades, but comprises well-structured dynamic patterns that are pivotal for the functioning of brain circuits. Spontaneous activity appears early during embryonic development and participates in the formation and interconnectivity of the developing neuronal circuits. At early postnatal stages, the combination of evoked activity (from sensory inputs) and spontaneous activity refines the young neuronal circuits to master complex tasks, such as motor coordination and the processing of visual information. In the adult brain, spontaneous activity takes part in input selection, information processing, memory consolidation and retrieval, and several other actions [6].

Despite continuous advances, the mechanisms that initiate and maintain spontaneous activity in neuronal circuits are still poorly understood, both from a physiological perspective and in modeling scenarios. However, circuits as diverse as the retina, the spinal cord, the cortex, thin slices of brain tissue, and the cultures of dissociated neurons described above exhibit some sort of sustained spontaneous activity patterns. This hints at the existence of universal mechanisms that robustly drive any neuronal network towards the generation of these structured, spatiotemporal, spontaneous discharges. To tackle this elegant and important neuroscience paradigm, different scales and systems are being investigated, from measurements and modeling at the brain level to more accessible and controllable in vitro preparations in the form of neuronal cultures.

In the human brain, spontaneous activity is often referred to as the resting state [9], i.e., the basal activity in the brain in the absence of stimuli and the conductance of specific tasks. Recent studies, particularly those led by G. Deco in Barcelona, have shown that the resting state exhibits a series of properties that reflect pivotal aspects of the functioning of the brain and its complexity [9,10]. These studies have been framed in the context of a non-linear physical model to unveil its key elements and mechanisms [8]. The model considers a set of brain areas, each of them formed by an ensemble of interconnected excitatory and inhibitory neurons and whose dynamics follow realistic IF descriptions. The dynamics in each single brain area are completed with a background noise that stimulates activity in the neuronal population and whose structure is similar to the one observed in actual brain measurements. The non-linear nature of the IF model and the coupling between neurons and the noise ultimately settles the dynamics of each brain area in a stationary "attractor" state. The different brain areas are then interconnected following real, precisely measured structural maps and the dynamics of the entire "brain" are then investigated as a function of the coupling strength between brain areas. This model shows that when the coupling is too weak, different brain areas fire independently in a low-firing regime. When the coupling is too strong, the entire brain fires synchronously, i.e., in an "epileptiform" manner with no spatiotemporal structure. For intermediate couplings, a multi-stability scenario emerges, characterized by the coexistence of many attractors, each of them a focus of high neuronal activity. This activity propagates across different areas in the brain, ultimately shaping a resting state with a rich spatiotemporal structure and a functional connectivity that is very similar to the one measured in humans.

This study by Deco's group is not only elegant from a physical perspective, but also enlightening in its neuroscience implications. First, the resting state is tightly linked to the structural connectivity of the brain, so that strong variations in the repertoire of activity patterns indicate important circuit anomalies, for instance due to disease. Second, the spatiotemporal structure of the resting state emerges as a subtle interplay between three key elements, none of them expendable: brain circuitry, local neuronal dynamics, and noise. Hence, the resting state reflects the dynamic capabilities of the brain and its capacity to respond to stimuli.

Noise focusing: addressing spontaneous activity in cultures

A complementary approach to understand the mechanisms that initiate spontaneous activity is being pursued by our group in Barcelona, in studies of neuronal cultures. One major aim is to elucidate the physical basis of the emergence of coherent spontaneous activity in relatively simple and controlled networks [26]. Specifically, we have studied the transition from the completely random firing of neurons at early stages of culture development to the first signature of coordinated collective action, in the form of synchronous and almost periodic bursts of activity by the entire network (Figs.

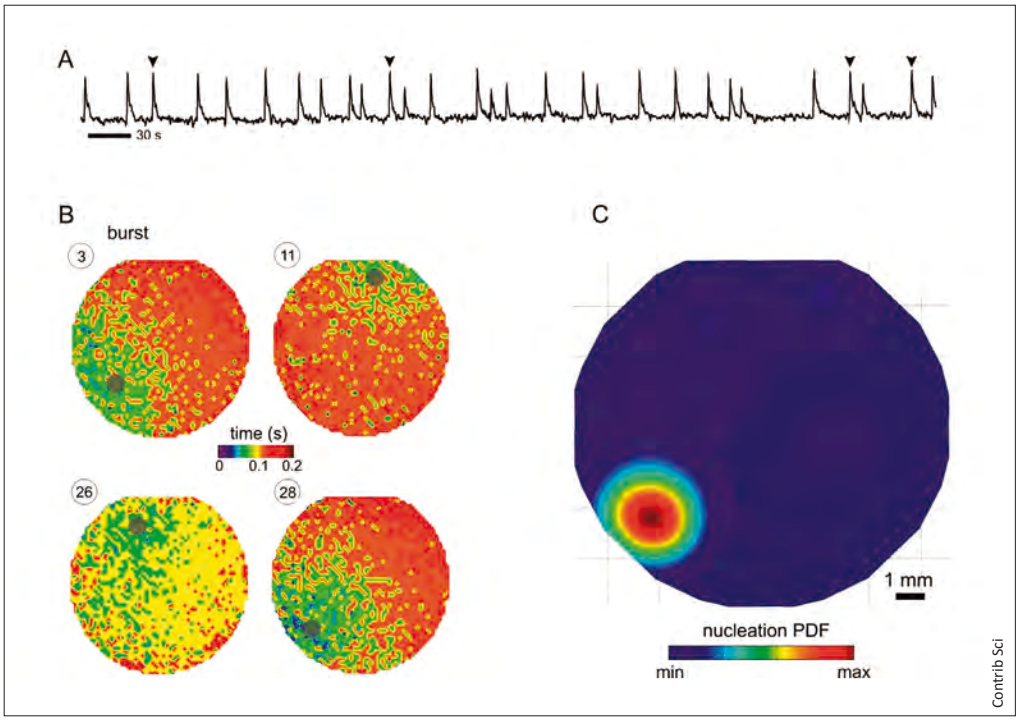

Fig. 3. Initiation and propagation of activity in homogeneous neuronal cultures. (**A**) Typical average fluorescence trace of a neuronal culture, limited to the first 10 min of recording. The recording corresponded to a circular culture 13 mm in diameter containing on the order of 100,000 neurons. The trace shows the average signal of all the neurons and illustrates the existence of regimes of high coherent activity (bursts, peaks of fluorescence) combined with intervals of almost no activity. The bursts marked with arrowheads are those analyzed in (B). (**B**) Examples of the initiation and propagation of activity in the same culture. The encircled values indicate the burst number along the recording. The color plots show the propagation of activity, which approaches a circular wave advancing at 50–60 mm/s. The gray circles mark the region where the burst initiated. Bursts #3, #11, and #26 initiated in completely different areas. Bursts #3 and #28 essentially started at the same location and displayed almost identical characteristics, which were also shared by ~80% of the recorded bursts. (**C**) Probability distribution function of initiation, highlighting the existence of a strong focus of activity at the bottom-left corner, i.e., the area where activity initiated in most of the cases. (See [26] for a detailed explanation).

2B and 3A). This phenomenon is very robust but nevertheless defies intuition, as it is a self-organized process that functions without the guidance of an internal clock or pacemaker, not even the presence of specialized leader neurons coordinating the process, as proven by computer simulations with identical neurons mimicking the cultured networks. The mechanism that enables the spontaneous generation of this coherent pulsation of large numbers of randomly connected neurons subject to random firing was recently discovered [26] through experiments using calcium imaging techniques in neuronal cultures, combined with a detailed in silico model of simulated networks.

The first important experimental observation in our study of neuronal cultures was that the global bursts were mediated by the fast propagation of an excitation wave through the culture that had not been resolved before (Fig. 3B). Once the waves were resolved in space and time, we observed that they were always initiated at a few localized spots, characteristic of each culture (Fig. 3C). The striking observation was that the nucleation of waves at different spots occurred in a completely random sequence, even though the bursts occurred nearly periodically in time. The phenomenon was thus noise-driven, but the period between bursts had to be fixed by an intrinsic time scale of recovery of the synaptic connections. These results were explained on the basis of a new mechanism that we called “noise focusing.”

The basic idea is that in a network of IF elements, the spontaneous (random) firing of neurons propagates its influence through the network connections such that it is strongly amplified both by the nonlinear dynamics of the network nodes and by the multiplicity of paths that connect two neurons. A single spontaneous firing may thus induce a cascade of activity in a group of neurons. The result is that the network endows the background activity of spontaneous firing with a nontrivial spatiotemporal structure that is not simply related to the specific connectivity of the network but

involves a complex interplay between topology and dynamics. The background activity is composed of the superposition of avalanches of activity of all sizes following a power law distribution. A careful statistical analysis of this structured background activity reveals an effective functional network with a scale-free degree distribution, even though the structural network has a Gaussian degree distribution [26].

Within this framework, the selection of nucleation spots is the result of highly inhomogeneous and anisotropic mechanisms of noise amplification introduced by the IF dynamics in a highly clustered network. This local amplification is very sensitive to the detailed wiring of the network. The nucleation sites can then be seen as the sinks of the averaged noise flow, that is, those points at the confluence of paths of high noise amplification. The a priori homogenous primary source of noise, that is, the spontaneous random firing of the neurons, is propagated and amplified, resulting in a strongly localized concentration of noise-generated activity at some specific spots. This spatiotemporal concentration of the background activity is what we call "noise focusing." As a basal physical phenomenon this should be generically present in neuronal networks unless specific regulation or other, stronger effects are taming, shaping, or preventing this activity. Moreover, this model nicely illustrates how physical phenomena can shape a situation in which biological blueprints must adapt and specific biochemical and genetic regulation must operate to build up the complex architecture of real neuronal tissues.

Conclusions

The comprehension of the human brain has long fascinated humanity as much as the structure of Nature and its governing laws. However, what initially began as an exclusive task of neurobiologists has evolved in just a century towards a highly interdisciplinary field of research in which Non-linear Physics and Network Science are major contributors. These branches of knowledge have provided a wealth of resources and modeling tools that, together with relatively simple experimental systems, have revolutionized our vision of the main agents that shape the dynamics of the brain and its exquisite complexity. The concepts presented in this brief review are just a fraction of all the potential that Physics can offer to studies of brain function. Indeed, if the 21st century is to be the "century of the brain," we firmly believe that Physics will play a central role in it.

Competing interests. None declared.

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